1973 MICROBIOLOGY REPORT

ON SUDBURY ENVIRONMENTAL STUDY

PART A - LAKE RECLAMATION STUDY

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SUMMARY

A preliminary microbiological study was undertaken during the summer and fall of 1973 to determine the effects of reclamation (lime treatment) of selected acidic lakes near Sudbury, Ontario on microbial populations in these lakes.

The pH of the two lakes selected for treatment, Middle and Lohi rose to 7 - 7.5 after lime treatment, while the pH of the control lakes, Hannah and Clearwater remained at the initial value for all lakes of 4 - 4.5.

The population of aerobic heterotrophic bacteria (SPC) increased markedly in Middle and Lohi Lakes within 3 weeks after lime treatment to 150,000 and 14,000/100 ml respectively and remained at these relatively high levels throughout the study period. The median SPC's for the control lakes which were similar to those of the corresponding treated lakes prior to liming, did not show an increased SPC. Median SPC's for Hannah and Middle Lakes respectively were only 440 and 1400/100 ml.

Increasing the pH of the lake water had no effect on yeast and mould populations, although yeast counts declined in all lakes with the onset of colder conditions

in the fall. Yeast counts for all Sudbury area lakes were moderate, median values ranging from 2 - 100 c.f.u.* per 100 ml and consisting predominantly of the genera Rhodotorula, Cryptococcus, and Aureobasidium. Qualitatively and quantitatively, the yeast flora was considered indicative and normal for oligotrophic waters.

In acid lakes, the decomposition of organic substances is diminished. The "liming effect", whereby the heterotrophic bacterial count in the water column of the treated lakes increased significantly after liming, appeared to be a result of increased bacterial metabolism of soluble organics at a more favourable pH. Decreased concentrations of Cu and Ni in the limed lakes after treatment would alleviate possible toxic or inhibitory effects of these metals on the microbial populations as well. The heterotrophic bacterial population appeared to be aciduric, i.e. survive the pH of 4 - 4.5 in the acidic lakes, but proliferation at the lower pH was reatarded.

Acidic lake conditions were unfavourable for sulfate-reducers and non-acidophilic, autotrophic sulfur-oxidizing bacteria (Thiobacillus thioparus group). Populations of these bacteria were lower in the acid control lakes (Clearwater and Hannah) than in corresponding neutral pH

* colony-forming units

lakes (Lohi and Middle). However, counts of acidophilic, autotrophic sulfur oxidizers (T. thiooxidans) were not significantly different between control or treated lakes, but were significantly greater in the acidic Sudbury lakes than in MacLean Lake, a neutral pH eutrophic lake in Simcoe County, selected for comparison with the dystrophic Sudbury area lakes. The non-acidophilic sulfur oxidizers were more numerous in the water column of MacLean Lake than in the acidic Hannah and Clearwater Lakes.

It was revealed that the median total coliform (TC) count of 210/100 ml in MacLean Lake was significantly greater that TC counts of <4/100 ml in the reclamation lakes. It appears that conditions for growth and survival for the Enterobacteriaceae are poor in the dystrophic study lakes.

Failure to detect the sensitive ammonia oxidizing autotrophic bacteria (Nitrosomonas spp.) in any of the Sudbury lakes, further supported the dystrophic term applied to the reclamation lakes where retarded cycling of nitrogen was occurring.

It was also observed that the median standard plate count (SPC) was significantly greater in MacLean Lake (7300/100 ml) than in the control lakes, Hannah (500) and Clearwater (1000), but no significant difference

in SPC was noted between MacLean and Middle or Lohi lakes after liming.

The treated lakes may come back into production and sustain a fishery if environmental conditions are made favourable for all trophic levels of the ecosystem, including the microbial decomposer group, important in recycling of nutrient elements from the sediments to stimulate primary production.

Future studies should concentrate on the activities of major segments of the microflora, particularly in mobilization of C, N, P and S compounds from the lake sediments subjected to reclamation treatments.

GENERAL INTRODUCTION:

Continuous smelter operations over the past few decades have proven detrimental to the terrestrial and aquatic ecosystems of the Sudbury region. At present, with the increased height of the large INCO stack (1250 '), the emitted pollutants are carried further afield by prevailing air currents up to and exceeding 100 miles, where they are deposited in more dilute form on the earth's surface by atmospheric precipitation. Prior to the introduction of the large stack, the bulk of the pollutants were deposited within the Sudbury region where damage was visibly manifest on the ecosystem. The principal pollutants emitted by the smelters are sulfur in the form of SO_2, H_2SO_4 and S°, and to a lesser degree oxides of heavy metals, iron, copper, nickel, arsenic and traces of other metals.

Numerous lakes near Sudbury have become acidic and declining fisheries are an unfortunate result of this condition. Elevated levels of dissolved metals add to the difficulty of sorting out the factors responsible for changes in fish species and numbers.

It has been established that considerable variability exists in the water quality of the lakes. The effects of SO_2, sulphuric acid and other airborne

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contaminants to lake systems are considerably modified by natural factors such as background geology, size and depth of lake, watershed area and presence or absence of inflows and outflows.[1]

The primary objectives of the preliminary investigations during 1973 were as follows:

1. A population dynamics approach was emphasized to gain background information on the quantity and quality (diversity) of aquatic microorganisms of significance to the carbon, sulfur and nitrogen cycles in the Sudbury lakes.
2. To determine levels of heterotrophic microorganisms, especially those which have adapted to growth under acid conditions, i.e. acidophilic bacteria, yeasts, moulds and to assess their potential role in the mineralization of organic matter in acidic and non-acidic lakes. A comparative characterization of lakes as to the significance of various genera and species of yeasts present is under investigation.
3. To determine if any factors are responsible for retarding growth and activities of the

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1. Personal communication, Nels Conroy, MOE Biologist, Sault Ste. Marie.

microbiota involved in the natural cycling of nutrients and decomposition processes in the lakes.

4. To detect any outstanding changes in the numbers and types of microorganisms in the Reclamation Lakes as a result of liming treatments and to relate these changes in terms of water and/or sediment chemistry.

Numerous studies have been conducted on the ecology of microorgansisms in surface waters affected by acid mine wastes. These include the well-known autotrophic acidophilic iron and sulfur bacteria (Thiobacillus - Ferrobacillus) frequently associated with mineral wastes from the mining industry. Most of these studies have been concerned with streams and lakes contaminated by direct surficial run-off and leaching from mine-mill tailings, or exposed mineral bodies of strip-mines. In the case of the Sudbury area lakes, the acidic mineral pollutants are similar (e.g. heavy metals, sulfuric acid). These have reached the body of water conveyed by air directly to the watershed in question.

In acidic streams (pH 2.5) polluted by drainage from strip mines in Kentucky, the predominant microbial

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forms apart from the acidophilic autotrophs (sulfur and iron oxidizers) are yeasts and moulds (Ehrlich, 1963; Weaver & Nash, 1968). The latter mentioned authors found fewer heterotrophic bacteria in an acid-polluted stream (pH 3.5) than in a connecting unpolluted branch (pH 6.5) (10,000 - 200,000 / 100 ml compared to 400,000 - 5 X 10^6 / 100 ml).

Dugan and Randles (1968) found a decrease from over 500,000 / 100 ml at pH 6.6 to 1500 / 100 ml at a pH of 2.7 in a study of aerobic heterotrophic bacteria in an acid-polluted stream. The predominating microbes found in the acid waters were yeasts and moulds along with gram-negative bacteria of the genera, Achromobacter and Pseudomonas. Rhodotorula and Trichosporon are yeasts commonly associated with acid polluted streams (Ehrlich, 1963).

Tuttle et al. (1968) postulated that the dispersed heterotrophic bacteria recovered from an acid stream were transients that entered the stream via run-off or from non-acid contaminated streams. Their findings corroborated Dugan & Randles (1968) work, and reported, in addition the virtual absence of gram positive bacteria in the acidic stream. A distinct survival advantage

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of gram negative to gram positive bacteria is attributed to a greater permeability barrier to H^+ ions by the lipopolysaccharide cell wall of the former group.

METHODS:

The bacteriological samples were taken to correspond as closely as possible with the regular routine sampling by personnel of the Water Quality Branch. In the Intensive Monitoring Programme, three stations per lake were monitored and in the Restoration Study, one station on each of the four lakes as selected by Water Quality Branch were sampled. Bacteriological analysis for the early summer survey was conducted in the mobile laboratory at Mac Farlane Lake. Subsequent samples were packed on ice and shipped to the Toronto laboratory for analysis next day.

Water samples were taken from one meter below the surface in sterile plastic bottles, and 1 meter from the bottom by means of a rubber bulb sampler. Sediment samples were taken occassionally using an EcKman dredge.

Water and sediment samples were analyzed for the following microbiological parameters: Total aerobic heterotrophic bacteria, acidophilic bacteria, yeasts and moulds, sulfate-reducing bacteria, sulfur oxidizing bacteria and occasionally nitrifying bacteria and the routine sanitary parameters.

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The populations of sulfur and nitrifying bacteria in water and sediment were enumerated by use of an MPN (most-probable-number) method with a 3-tube series for each dilution. The medium used to detect the presence and levels of sulfate reducing bacteria was API broth with tryptone (formula described in Difco Manual). The $Fe(NH_4)_2(SO_4)_2$ and ascorbic acid solutions and an iron nail were aseptically added to each tube at the time of inoculation with the sample. Test tubes were incubated anaerobically at room temperature for 3 weeks, at which time the number of positive tubes showing SO_4-reduction (blackening of broth) was recorded. Estimation of the number of sulfate-reducers per 100 ml of water or per gram of sediment (wet wt.) was made by reference to a standard MPN statistical table.

For the sulfur-oxidizing chemolithotrophic bacteria, mineral salts medium containing sodium thiosulfate was used according to the formula described by Postgate (1966), Journal of Laboratory Practice 15: 1239. The pH of the media for the acidophilic sulfur oxidizers (Thiobacillus thiooxidans) was 4.5 and for the non-acid tolerant sulfur oxidizers (T.thioparus group), was set

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at 7.2. Sterile control test tubes of broth were incubated along with the inoculated MPN tubes to check on chemical oxidation of thiosulfate. All tubes were incubated at room temperature for 4 weeks. At the end of 4 weeks, all tubes were examined for sulfur oxidation by determining the pH and sulfate concentration of each tube according to an approximate procedure. The pH of the contents of each tube was determined quickly using sensitive pH indicator paper. An approximation for $SO_4^=$ concentration in each tube was made by adding 1 ml of contents of each t.t broth media to a large t.t. containing 18 ml distilled H_2O. One ml of 2% $BaCl_2$ solution was then added, contents shaken and allowed to stand for 30 seconds for development of turbidity for each tube was readily measured by a visual comparison to a sterile control. If the pH of the inoculated broth had dropped below the control or if the turbidity was greater than the controls, a positive test for bacterial thiosulfate oxidation was assigned that tube.

The ammonium mineral salts broth used for detection of autotrophic ammonia oxidizers (Nitrosomonas) had been described in a previous OWRC report, (1969)

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(Bacteriology of Little Round Lake, Ontario, F.R. Thompson) Incubation of nitrifier broth was carried out at room temperature for 4 weeks. At the end of the incubation period, a spot test to detect presence of nitrite and/or nitrate in each t.t. was employed using diphenylamine reagent.

Populations of heterotrophic microbes were determined by membrane filtration (MF) methods. Total Plate Count (TPC) of aerobic heterotrophic bacteria was determined by placing Millipore membranes (0.45 μ) onto agar plates of Tryptone-glucose-yeast extract (TYE) media and incubating at 20°C for 3 days.

For acidophilic or acid-tolerant bacteria, TYE agar with pH adjusted to 4.8 was used. Incubation at 20°C was prolonged in this case to 8 days. Sanitary bacteriological parameters (total coliform, fecal coliform and fecal streptococcus) were determined occasionally throughout the survey.

Fungal populations (yeasts and moulds) were determined using yeast-malt extract agar (YM), acidified to pH 4.5 (formula described in Difco Manual). Later, a pH 4.0 was used routinely for eliminating all acid-

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tolerant bacteria tending to grow on YM media. This means of separation of the two major groups of microbes was preferred to the use of antibiotics which gave less than perfect inhibition of bacteria. The plates were incubated at 20°C for at least 3 days before counts were made. A temperature of 5° - 10°C for a period of up to 20 days gave slightly higher yeast counts partly due to the retarded growth of spreading molds, e.g. Trichoderma viride, commonly present in lake water samples.

Transfers of yeast colonies were made to culture tubes of YM agar. After incubation, they were refrigerated until taxonomic determinations could be made.

For identification of yeasts, morphological and physiological characteristics were determined as recommended by Wickerham (1951) and Lodder (1970). Morphological determinations consisted of: colony and streak characteristics on Wickerham morphology agar; cell size and shape; ballistospores present or absent; ascospores present or absent. Physiological determinations consisted of: the ability to utilize nitrate as a nitrogen source; the ability to grow on vitamin free medium or with added thiamine; the ability to assimilate carbon compounds: maltose, lactose, raffinose, melibiose, melezitose, erythritol, inositol, starch and sucrose.

PART A - LAKE RECLAMATION STUDY

Four lakes in the immediate vicinity of Sudbury which had appreciable concentrations of heavy metals and acid pH conditions were selected for study. These lakes were located Southwest of the city of Sudbury and south of the Copper Cliff smelting complex. Hannah and nearby Middle Lake constituted one pair for comparison while Clearwater and Lohi Lakes made up the other two. The first two lakes were located about 5 km from the smelter, the latter pair about 11 km from the smelter. Hannah and Clearwater Lake served as control lakes while Middle and Lohi were chosen for treatment. The pH of all lakes prior to treatment with calcium hydroxide was 4 - 4.5. After liming, the pH of Middle and Lohi Lakes rose to 7 - 7.5 and remained in this range throughout the fall season.

Results and Discussion:

Microbial counts for all samples were tabulated as shown in the Appendix tables. Statistical comparisons were made to determine whether or not each parameter showed a significant difference according to both depth of sample (surface vs depth) and season (summer vs fall), for each lake individually. Then

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the populations for each microbial parameter in water column and sediment were compared between Lohi (treated) and Clearwater (control) Lakes and between Middle (treated) and Hannah (control) Lakes to determine if liming had any significant effect on levels of the aquatic population. Because of the scarcity of data for certain parameters, a non-parametric test of significance was used to treat the data. In the Mann-Whitney U-test employed, each piece of information was given a rank and two separate groups of data were then analyzed to determine if one group was significantly different from the other.

An effort was made to assess differences in certain segments of the microbial ecosystem between the oligotrophic Sudbury Lakes studied and MacLean Lake (Simcoe County), a eutrophic lake with a neutral pH and lower content of the heavy metals, Cu and Ni. MacLean Lake lies over Precambrian bed-rock but is a more productive lake with higher nutrient and plankton levels than the oligotrophic-dystrophic Sudbury area lakes studied.

A quantitative comparison of the various segments of the microbial population between Middle and Hannah Lakes is shown in Table I. The parameter which

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MICROBIOLOGICAL ASPECTS OF LAKE RESTORATION

TABLE I Quantitative comparison of various microbial parameters between Middle and Hannah Lakes

Median Count per 100 ml H_2O

PARAMETER	MIDDLE LAKE	HANNAH LAKE
SPC	150,000	440 *
YEASTS	28	30
MOULDS	9	16 *
SO_4-Reducers	4	<3
Thiobacillus (I)[1]	<3	9
Thiobacillus (II)[2]	460	4 *(p=.05)
Nitrifiers	<3	<3
Total Coliform (TC)	<4	<4
Fecal Coliform (FC)	<4	<4
Fecal Streptococcus (FS)	<4	<4

Median Count per gram sediment (W.W.)

SPC	160,000	92000
YEASTS	20	9
MOULDS	300	54 *
SO_4-Reducers	930	<30 * (p=0.1)
Thiobacillus (I)	90	930
Thiobacillus (II)	46000	430 * (p=.1)
Nitrifiers	<30	<30
Total Coliform (TC)	<100	<100
Fecal Coliform (FC)	<100	<100
Fecal Streptococcus (FS)	<100	<100

Mann-Whitney U-test used in data analysis.

1 - Acidophilic Sulfur oxidizing bacteria
2 - Non-acidophilic Sulfur oxidizing bacteria
* - SIGNIFICANT DIFFERENCE (p=.025 except where otherwise specified)

showed the greatest difference between the lakes after liming was the aerobic heterotrophic bacterial count (SPC). Middle Lake was sampled only on two occassions before liming, but the SPC in surface and bottom water samples in Middle Lake before liming was about the same order of magnitude as in Hannah Lake. The drastic increase in total aerobic heterotrophs occurred in early October (about 12 days after initial liming treatment) and remained in the order of 10^5 / 100 ml throughout the October - November sampling period. The median count per 100 ml water for aerobic heterotrophic bacteria of 150,000 in Middle Lake was significantly greater than 440 in Hannah Lake. Yeasts and moulds are heterotrophic fungi which normally are able to grow well under low pH conditions, provided adequate nutrients, organic matter and other environmental factors are favourable. The levels of yeasts (30 cells / 100 ml) and moulds were not very high and no significant difference was found in yeast count between the two lakes. The mould counts of 16 / 100 ml in Hannah Lake was almost twice that obtained for Middle Lake and was significantly different. The only other parameter which showed a significant difference between the lakes was the non-acido-

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philic group of sulfur-oxidizing bacteria, the T. thioparus group (II). A median count of 4 cells / 100 ml for Hannah Lake contrasted to 460 / 100 ml for the treated Middle Lake was significant at the 95% level of confidence. This group of autotrophic sulfur-oxidizing bacteria would not grow in the acid waters of Hannah Lake (pH 4 - 4.5), but could oxidize reduced sulfur compounds in Middle Lake where the pH was around neutrality. However, the acidophilic sulfur oxidizers of the Thiobacillus thiooxidans group (I), were undetected in Middle Lake (<3 / 100 ml) and only 9 / 100 ml in Hannah Lake. This low count in Hannah Lake would suggest a lack of suitable reduced sulfur for growth substrates for the acidophilic S oxidizers in Hannah Lake. Perhaps some sulfide was being released from the sediments of Middle Lake after liming to account for higher levels of the T. thioparus group in the water column. Sulfate-reducing bacteria increased in Middle Lake sediment after liming (median count 930 / g), while none were ever detected in Hannah Lake sediment (<30/g). In the water column, these anaerobic bacteria were hardly detected (<3 and 4 per 100 ml) in the pair of lakes. The Eh of the water column was too high

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to permit anaerobic growth of sulfate-reducers.

Nitrifying bacteria, i.e. the autotrophic ammonia oxidizing bacteria were not found in the water or sediment of either lake in significant levels. These bacteria depend on a source of ammonia for their growth and energy requirements and are acutely sensitive to acidity and heavy metals, especially copper. Loveless & Painter (1968) reported on inhibition of Nitrosomonas sp. by Cu^{+2} at a concentration of .05 mg/l. Zinc, cobalt and nickel were more toxic to bacteria in the presence of traces of copper than alone (synergistic toxic effect). Thus low levels of free NH_3, low pH and presence of heavy metals may account for the scarcity of the nitrifying bacteria in these lakes.

The sanitary parameters, (coliforms and fecal streptococci) were generally absent from the water column and sediment of the lakes, (<4/100 ml and <100/g sediment).

In lake sediments, the total aerobic heterotrophic populations were moderate (10^5/g) and showed no variation between Middle and Hannah Lakes. Moulds were more numerous than yeasts. The former parameter was significantly greater (300/g) in Middle Lake

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than Hannah Lake (54/g) sediments. The non-acidophilic sulfur oxidizers were found in higher levels (46 X 10^3/g) in Middle Lake than Hannah Lake (430/g). Conditions may have not been favourable for growth of the non-acidophilic sulfur oxidizers in the acidic sediments of Hannah Lake. Since sulfate-reducing bacteria were detected in Middle Lake sediment and not in that of Hannah Lake, the lack of reduced sulfur substrates may also have been a factor in precluding growth of the sulfur-oxidizers in Hannah Lake sediment.

The Microbiological data for Lohi and Clear-water Lakes is compared in Table II . Sampling was done from August to November, before and after the initial liming of Lohi Lake on October 17. The counts of aerobic heterotrophic bacteria (SPC) in the water column of Lohi and Clearwater Lakes were not significantly different, but after liming, the total plate count increased in Lohi Lake to a median value of 14,000 / 100 ml from 1200 / 100 ml before, while no corresponding change occurred in Clearwater Lake. This observed difference after liming was significant. Yeasts and moulds which were found in much lower number than bacteria

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TABLE II - Quantitative Comparison of Microbiological Data for LOHI and CLEARWATER LAKES.

Median Count per 100 ml H_2O

PARAMETER	LOHI (limed)	CLEARWATER (control)	
TPC (before Oct. 17)+	1200	1400	
(after Oct. 17)	14,000	750	*
Yeasts (before)	44	110	
(after)	2	3	
Moulds (before)	14	12	
(after)	6	2	
SO_4-Reducers	150	<3	*(p=0.1)
Thiobacillus (I)	4	6	
Thiobacillus (II)	43	4	
Nitrifiers	<3	<3	
TC (Total Coliform)	<4	<4	
FC (Fecal Coliform)	<4	<4	
FS(Fecal Streptococcus)			

Median Count per gram sediment (wet wt.)

TPC	270,000	140,000	
Yeasts	<10	<10	
Moulds	100	120	
SO_4-Reducers	4300	40	*(p=0.1)
Thiobacillus (I)	2400	430	
Thiobacillus (II)	7800	870	
Nitrifiers	<30	<30	
TC (Total Coliform)	<100	200	
FC (Fecal Coliform)	<100	<100	
FS (Fecal Streptococcus)	<100	<100	

* SIGNIFICANT DIFFERENCE
\+ date of initial liming

TABLE III - Comparison of Microbiological Data for Each of LOHI and CLEARWATER LAKES before and after liming treatment.

Median count per 100 ml H_2O

PARAMETER	LOHI (limed)		CLEARWATER (control)	
	before	after	before	after
TPC	1200	14,000	1400	750
Yeasts	36	2 *	100	3 *
Moulds	14	6	12	2
SO_4-Reducers	150	<3	<3	<3

* Significant Difference

showed no significant difference between the two lakes, either before or after the time of lime treatment.

However, in both Clearwater and Lohi Lakes, the yeast count in the water column declined significantly after October 17 compared to earlier surveys. (Table III) This phenomenon, apparently unrelated to pH changes, occurred in other lakes in the fall and may be related to a decline in water temperature.

A significant increase in SPC was observed in Lohi Lake after liming but not in Clearwater Lake (Table III). Sulfate-reducing bacteria were never detected in Clearwater Lake water but were present in Lohi Lake (September) even before liming. No significant change in the population of sulfate-reducing bacteria occurred from treatment. The sulfate-reducers were detected in sediment of both lakes but the median count for Lohi (4300/g) was significantly greater than the levels of these anaerobes in Clearwater Lake sediment (40/g). The higher levels of sulfate-reducers detected in the water column of Lohi Lake, 1 meter from the bottom, coincided with anoxic conditions in the hypolimnion and presence of H_2S first noticed in September. The inherently higher pH

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of Lohi Lake sediment even before liming began (pH 6.0) compared to sediments of Clearwater (pH 5.4), Hannah (4.5) and Middle (4.8) lakes may account in part for the higher activities of the sulfate-reducing bacteria in the former lake sediment. The free NH_3 and total N of 0.5 and 1 ppm respectively for the bottom water of Lohi Lake was higher than the levels of N at the surface (0.09 ppm NH_3 and 0.23ppm Total N) (Appendix Table 1). The other lakes did not show these higher levels of ammonia in the water column, 1 m from the bottom which probably resulted from greater activity of ammonifying microbes in the Lohi sediments.

Both groups of sulfur oxidizing bacteria were at low levels (4 - 43 / 100 ml) in the water column and more concentrated in sediments (430 - 7800/g), but the counts of these bacteria between Lohi and Clearwater Lakes were not significantly different.

As in the other Sudbury lakes studied, the ammonia oxidizing bacteria were not detected in water or sediments. This was also true for total and fecal coliforms and fecal streptococci. The fecal-associated bacteria do not appear to have entered the lake in great numbers from cottages or shore-line surficial run-off, or if they

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have, these bacteria have not found the low pH and poor nutrient status of the water conducive for survival and have died off.

In comparing the bacterial populations at the deep water central station of MacLean Lake (eutrophic) to the populations in the four study lakes (Tables IV and V), it was found that the median total coliform (TC) count of 210 / 100 ml in MacLean Lake was significantly greater than the TC counts of <4/100 ml in the oligotrophic Sudbury lakes. However, populations of fecal coliforms (FC) and fecal streptococci (FS) were not significantly different (<4/100 ml) between lakes. Conditions for growth and survival of the family Enterobacteriaceae may be poor in the dystrophic lakes studied.

The standard plate count (SPC) of aerobic heterotrophic bacteria was significantly lower in the water column of the untreated lakes, Hannah and Clearwater (500 and 1000 per / 100 ml respectively) than in MacLean Lake (7300 per 100 ml). No significant difference in SPC between MacLean Lake and the lime-treated lakes,

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Middle and Lohi was observed in both water column and sediments. Aerobic heterotrophic bacteria were in the range 10^5/g sediment for all lakes and no significant differences were apparent.

As with the TPC parameter, the counts of sulfate-reducers, and non-acidophilic sulfur-oxidizers (II) were significantly lower in the two control lakes, Hannah and Clearwater than in MacLean Lake, while the acidophilic sulfur-oxidizing bacteria (I) were more numerous in the acid control lakes than in MacLean Lake. The acid pH conditions below pH 4.5 in the control lakes would not be favourable for growth of sulfate reducing or the non-acidolphilic sulfur-oxidizers. Only the acidphilic Thiobacillus thiooxidans would be active in the acid lakes. However, the T. thiooxidans levels in the water column of all lakes were relatively low compared to T.thioparus except for the acid control lakes where the former group dominated. In sediment, no significant differences were indicated between MacLean or the four Sudbury lakes with respect to levels of T. thiooxidans. The levels of T. thioparus in Middle Lake sediment (4600/g) were greater than the population in MacLean sediment (210/g).

Nitrifiers showed no significant variation in water columns between MacLean and the other lakes (<2/100 ml), but the levels of the ammonia oxidizers were greater in

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TABLE IV - Comparison of Microbial Populations of RECLAMATION LAKES with eutrophic MACLEAN LAKE

Median count per 100 ml H_2O

PARAMETER	CLEARWATER	MACLEAN	LOHI
SPC	1000 *	7300	6800
Yeasts	50	10	24
Moulds	8	16	12
Sulfate-Reducers	<3 *	4	77
Thiobacillus (I)	4 *(p=0.1)	<3	6*(p=0.1)
Thiobacillus (II)	<3 *	240	43
Nitrifiers	<3	<3	<3
TC (Total Coliform)	<4 *	210	<4 *
FC (Fecal Coliform)	<4	<4	<4
FS (Fecal Streptococcus)	<4	<4	<4

Median count per gram Sediment (wet wt.)

SPC	140,000	210,000	270,000
Sulfate-Reducers	40	95	4300 (insufficient data for comparison
Thiobacillus (I)	650	230	2400
Thiobacillus (II)	870	210	4600
Nitrifiers	<30 *	930	<30 *
TC (Total Coliform)	<100	200	<100
FC (Fecal Coliform)	<100	<100	<100
FS (Fecal Streptococcus)	<100	<100	<100

* SIGNIFICANT DIFFERENCE

TABLE V - Comparison of Microbial Populations in HANNAH AND MIDDLE LAKES with MACLEAN LAKE

Median Count per 100 ml H_2O

PARAMETER	HANNAH	MACLEAN	MIDDLE
TPC	500 *	7300	100,000
Yeasts	30	10	42 *(p=0.1)
Moulds	16	16	9
Sulfate-Reducers	<3 *	4	4
Thiobacillus (I)	16 *(p=0.1)	<3	<3
Thiobacillus (II)	4 *	240	460
Nitrifiers	<3	<3	<3
TC (Total Coliform)	<4 *	210	<4 *
FC (Fecal Coliform)	<4	<4	<4
FS (Fecal Streptococcus)	<4	<4	<4

Median Count per gram Sediment (W.W.)

TPC	92,000	210,000	160,000
Sulfate-Reducers	<30 *	95	1500
Thiobacillus (I)	930	230	90
Thiobacillus (II)	430	210	4600 *(p=.05)
Nitrifiers	<30 *(p=.05)	930	<30 *(p=.05)
TC (Total Coliform)	<100	200	<100
FC (Fecal Coliform)	<100	<100	<100
FS (Fecal Streptococcus)	<100	<100	<100

* SIGNIFICANT DIFFERENCE

the sediment of MacLean Lake (930/g) compared to the oligotrophic lakes where none were detected (<30/g). As these ammonia-oxidizing autotrophs are generally associated with colloidal material (clays and organic matter), their very low populations in the water column are not surprising. The MacLean Lake sediment had a significant population of nitrifiers resident which is indicative of a eutrophic lake with recycling of nitrogen compounds occurring.

The absence of nitrifiers in the sediment (<30/g) of the oligotrophic Sudbury lakes may be due to the low sediment pH, presence of heavy metals, lack of free ammonia substrate or a combination of these factors. Under low pH conditions, ammonification in the sediments would be inhibited. More studies should concentrate on the nature of sediment organic matter in these lakes to determine the capacity for mobilization of organic-N, and complexed phophorus under more neutral pH conditions. As well, sediment respiration studies may lead to valuable findings on the effect of treatments on the O_2 uptake rates of sediment in a model system.

In view of the acid pH conditions of the Sudbury

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lakes, an attempt was made to enumerate levels of acidophilic bacteria, i.e. bacteria which prefer an acid pH for maximum growth or acid-tolerant bacteria which are capable of growing at acid pH conditions as well as neutrality. The standard heterotrophic plate count media (TYE) was pH-adjusted to 4.8 and membranes of filtered samples were incubated on this. However, it was found that yeasts were the predominant colonies which appeared after a few days aerobic incubation on this acidified medium. Relatively few bacteria showed up on the acidified TYE. However, the predominant bacteria which were cultured on the acidified TYE agar from the four lakes were yellow, and pink pigmented species of gram-negative bacteria, suspected as a member of the genus Flavobacterium. A few other acid-tolerant cream coloured colonies presently unidentified also appeared. On prolonged incubation at 15° - 20° C for a few weeks more bacterial colonies arose on membranes of acidified media in some cases, but overgrowth and competition by the faster growing yeasts prevented obtaining a count of acid-tolerant bacteria. Presently methods to inhibit yeast and mould growth on acidified

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TYE are being worked out to make possible an acid-tolerant bacterial count in the respective lakes studied.

Millar (1973) found the major microbial component of an acidic environment to be a gram negative acidophilic yellow pigmented rod which be named Flavobacterium acidurans. All acidophilic isolates were slow-growing under acid conditions, incubation requiring 1 - 4 weeks for colony formation (1mm diameter). The formation of mini-cells by Flavobacterium acidurans and also a strain of E.coli, was considered a cellular response to sub-optimal growth conditions.

In the water of neutral pH lakes, normally about 90 - 95 % of the bacterial population are found as gram negative rods principally of the genera Pseudomonas, Acinetobacter, Alcaligenes and Flavobacterium, the remaining 5 - 10 % as gram positive cocci or rods (Keeney, 1971). In acid-contaminated waters, the gram positive component of the aquatic microflora has been found in many studies to be absent (Millar, 1973; Thompson, 1972; Tuttle et al, 1968). No gram positive bacteria have yet been isolated on the TYE agar used to obtain the heterotrophic count in any of the acidic Sudbury lakes. This finding also supports the observations of other workers that

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gram negative bacteria have a survival advantage and greater tolerance to acid conditions than gram positive organisms.

Yeasts have been found in large numbers in acid surface waters and are believed to contribute appreciably to the degradation of organic matter in highly acid environments due to their high tolerance to hydrogen ions. (Thompson, 1972). The median counts were moderate, ranging from 10/100 ml in MacLean Lake to 60 in Hannah Lake, but these differences were not significant. In the acid lakes where pH ranged from 4 - 4.5, the yeasts would be considered to have a growth advantage over bacteria. The failure of yeasts to develop into larger populations in the oligotrophic lakes may be due to the scarcity of readily available organic matter and other inorganic nutrients in the water.

Water samples were also taken from a Beaver Pond near the road to Lohi Lake (see Table 8A for data). The water had a pH of 6.8 - 7.0 and a tea coloured appearance due to an apparent high humic acid content. Yeast counts in this eutrophic water approached 1000/100 ml, greater than levels observed for any of the lakes studied. These higher yeast counts may be a reflection of increased available organic matter.

In a study of gram negative bacteria of the family Enterobacteriaceae isolated from acid mine water, Rogers (1964) hypothesized that these bacteria were aciduric, and although they did not grow, they were not killed

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by the acid conditions. He found that yeasts metabolized best at a pH of 4.0 or lower, whereas the bacterial cultures metabolized little or not at all below a pH of 5.0 regardless of the substrate.

In another comparative treatise, Thompson (1972) found that substantial numbers of the yeast, Rhodotorula mucilaginosa (syn. rubra) survived and metabolized over a greater pH range than the gram negative bacterium, E. coli. Multiplication of Rhodotorula in nutrient media occurred at a lower pH range than did growth of E. coli. Both E. coli and R. mucilaginosa possessed sufficient endogenous nutritional reserves to permit these organisms to survive periods where nutrients were absent or unavailable to them. E.coli could metabolize glucose, though slowly at pH 3.5. The author concluded that if E. coli does survive this low pH, sufficient numbers of bacteria may persist and continue to grow actively when conditions become more favourable.

In the Sudbury lakes study, the heterotrophic bacterial population appears to be aciduric in that it is able to survive in the low pH environment of the acid lakes but proliferation at the low pH is retarded, as indicated by the poor ability of the majority of lake

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isolates to grow on TYE agar at pH 4.8.

More work will be conducted in 1974 to determine the capabilities of the microflora from both acid and non-acid lakes to proliferate and metabolize at acid pH conditions.

It is the contention of one of the authors (F. R. Thompson) that the natural aquatic bacteria present in the Sudbury lakes have not adapted to the relatively recent acid pH conditions. The physiological limit of pH for growth of most bacteria is around neutrality. Relatively few bacteria are acidophilic as compared to the fungi. This characteristic, i.e. growth preference at low pH took eons to evolve as part of the specific genetics of acidophilic microorganisms.

The "liming effect" whereby the aerobic heterotrophic bacterial count in the water column of the treated lakes, Middle and Lohi increased significantly after liming as opposed to no drastic changes in bacterial populations in the control lakes may be attributed to pH conditions becoming more favourable to the slowly metabolizing bacterial population. The preservative effects of acids are well known, as in the production

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of pickles, sauerkraut and silage. Also, the organic matter of acid peat bogs decomposes only very slowly. Organic matter in the water of the acid lakes may thus be relatively unavailable to the bacteria. When the pH of the water was increased to neutrality, the bacteria would then be able to assimilate and metabolize organic matter more rapidly, resulting in the increased bacterial biomass observed for Middle and Lohi Lakes.

At the higher pH in the dilute water environment, bacteria would be more competetive for organic matter than fungi.

It was also observed that generally for all lakes, the yeast population dwindled in the fall with the onset of cooler water temperatures. In the acid water, it would be expected that yeasts would metabolize organic matter more readily than bacteria. However, their growth rate and ability to metabolize organic matter may be reduced because of the nature of the substrates (bacteria have a more diverse diet), toxicity by heavy metals (sub-lethal effects of metals) or to the ability of the bacteria to more proficiently utilize the naturally low concentrations of organic matter in these dystophic -

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oligotrophic lakes.

As a result of lime treatment of Middle and Lohi Lakes, the concentrations of the principal heavy metals present (Cu and Ni), dropped appreciably. In these lakes, Cu and Ni concentrations of 0.5 and 1.1 ppm respectively decreased to 0.03 and 0.26 ppm after treatment. At the same time, the calcium concentration increased. Other workers have found that the toxic effects of metallic cations to microorganisms could be overcome by the addition of a proportional conentration of cations not usually toxic, e.g. Ca^{++} and Mg^{++} ions. Glucose metabolism was inhibited by Cu^{+2}, Fe^{+2}, Ni^{+2}, and Zn^{+2} but not by Ca^{+2}, Mg^{+2}, or Sr^{+2}. (Thompson, 1972). The lessened toxicity of heavy metals to aquatic microorganisms by liming is another factor favouring microbial growth in the treated ecosystem.

The treated lakes may come back into production and sustain a fishery if environmental conditions are made favourable for all trophic levels including the microbial decomposer group of the ecosystem. A beginning may in fact be in nutrient recycling from the lake sediments to stimulate primary production.

Results (Mycology):

As the yeast populations of the study lakes were reasonably distributed, an opportunity was afforded for a study of the taxonomic groups present in the lakes. For this purpose, colonies were picked from membranes and spread plates in reasonably large numbers to get an indication of the relative proportions of the different taxa present. The colonies were often mixed and further separation was required before biochemical tests could be carried out.

The data reported here were taken from samples collected from the lakes during September - November and transported to Toronto from Sudbury; the data is summarized in Table VI.

The red yeasts, mainly Rhodotorula spp. were consistently present in all samples. However, it was noted that when the yeast population was unusually elevated, the red yeasts accounted for 65% of the total. In addition, red-pigmented colonies were also species of Sporobolomyces, and an atypical species temporarily assigned to Bullera.

Determination of all yeast cultures to species have not been completed; however, the results show that the most numerous yeasts and those represented by the widest variety of species belong to the genera Rhodotorula and Cryptococcus.

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To date, six of the nine species of Rhodotorula have been isolated, and five taxa of Cryptococcus. These are: R. glutinis and R. rubra - both frequently isolated - and R. minuta var. minuta, R. pallida, R. piliminae, R. graminis, Cr. albidus var. albidus, Cr. albidus var. diffluens, Cr. laurentii var. laurentii, Cr. laurentii var. flavescens, and Cr. luteolus. Cr. albidus was the most commonly isolated species of Cryptococcus, although Cr. laurentii was also very numerous. (R. piliminae and R. graminis were not found in the intensive study lakes.)

Another frequently isolated organism was the yeast-like fungus Aureobasidium pullulans, known as the 'black yeast'. Some yeasts, tentatively assigned to the genera Candida and Torulopsis, were not numerous. Sporobolomyces roseus was isolated from the Reclamation lakes but was not found in the Intensive Study lakes. Similar results were found in the case of Hansenula and Pichia, which were isolated only sporadically.

The small sampling from MacLean Lake showed a small population of red yeasts but a much larger population of Cryptococcus. Rather strikingly, the soil organisms Hansenula and Pichia were well represented in this small sample, in contrast with their rarity in the Sudbury lakes. Eight out of

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TABLE VI

Yeast Distribution of Reclamation Lakes and MacLean Lake*

Genus/species	MIDDLE LAKE		HANNAH LAKE		LOHI LAKE		CLEARWATER LAKE		MACLEAN LAKE	
	Isolates	%	Isolates	%	Isolates	%	Isolates	%	Isolates	%
Non-ascospore Yeasts										
Rhodotorula										
glutinis	10	10.75	3	5.17	8	12.12	11	15.49	-	0.00
rubra	11	11.83	5	8.62	6	9.09	14	19.72	1	3.85
minuta var. minuta	3	3.23	-	0.00	1	1.52	-	0.00	1	3.85
piliminae	1	1.08	-	0.00	-	0.00	1	1.41	-	0.00
graminis	1	0.00	-	0.00	1	1.52	-	0.00	-	0.00
pallida	-	0.00	-	0.00	-	0.00	3	4.23	-	0.00
Cryptococcus spp.	41	40.9	29	50.00	34	51.52	29	40.85	10	38.46
Torulopsis (n.c.)**	-	0.00	-	0.00	1	1.52	-	0.00	-	0.00
Candida (n.c.)	2	2.15	1	1.72	2	3.03	-	0.00	3	11.54
Ascospore Yeasts										
Hansenula	-	0.00	1	1.72	-	0.00	-	0.00	4	15.38
Pichia	-	0.00	2	3.45	1	1.52	1	1.41	4	15.38
Ballistospore Yeasts										
Sporobolomyces	5	5.38	1	1.72	1	1.52	1	1.41	-	0.00
Bullera (n.c.)	1	1.08	-	0.00	-	0.00	-	0.00	-	0.00
Aureobasidium spp. (creamy white when isolated) - black yeasts	19	20.43	15	25.86	8	12.12	10	14.08	3	11.54
Others	-	0.00	1	1.72	3	4.55	1	1.41	-	0.00
TOTAL	93	100	58	100	66	100	71	100	26	100

* - eutrophic lake, for comparison

** - n.c. - not confirmed

TABLE VII

Yeast Distribution of Intensive Study Lakes

Genus/species	NELSON LAKE		FAIRBANKS LAKE		NELLIE LAKE		BASSOON LAKE	
	Isolates	%	Isolates	%	Isolates	%	Isolates	%
Non-ascospore Yeasts								
Rhodotorula								
glutinis	14	18.18	16	18.39	12	13.33	30	35.71
rubra	12	15.58	15	17.24	21	23.33	12	14.29
minuta var. *minuta*	1	1.3	2	2.3	3	3.33	-	
piliminae	-	0.0	-		-		-	
graminis	-	0.0	-		-		-	
pallida	-	0.0	1	1.15	-		-	
Cryptococcus spp.	34	44.16	33	37.93	46	51.11	35	41.67
Torulopsis spp. (n.c.)*	1	1.3	1	1.15	-		2	2.38
Candida spp. (n.c.)	4	5.19	0	0.00	3	3.33	3	3.57
Ascospore Yeasts								
Hansenula spp.	-		-	0.00	-		-	
Pichia spp.	-		-	0.00	-		-	
Ballistospore Yeasts								
Sporobolomyces spp.	-		6	6.9			1	1.19
Bullera spp. (n.c.)	-		5	5.75	-		-	
Aureobasidium spp. ('black yeasts')	8	10.39	6	6.9	5	5.56	1	1.19
Others	3	3.9	2	2.3	-	0.00	-	
TOTAL	77	100.0	87	100.0	90	100.0	84	100.0

* - n.c. - not confirmed

thirteen isolates of the genera Hansenula and Pichia were from sediment.

A preliminary comparison indicates that most yeast species studied are common to both water and sediment. To date, in this study, members of the genera Sporobolomyces and Bullera (?) have been found only in water.

Data collected on Middle Lake before lime treatment and, again, after lime treatment showed no significant differences in the variety of yeasts isolated. It appeared therefore, that the yeasts were not affected by the change of pH to neutrality.

An unusually large population of 'black yeasts' of 200 - 400 c.f.u./100 ml was present in Lohi and Clearwater Lakes in September. This ubiquitous fungus has both a terestrial and aquatic form. It is one of the predominant organisms in marine waters (Meyers et al, 1970) and is highly proteolytic.

Discussion (Mycology):

Investigators in various areas of the world have made studies of yeasts in natural bodies of water, both freshwater and marine. Some of these results are summarized below and it may be of interest to make some comparison with results

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obtained in the Sudbury lakes.

Quantitative studies (Van Uden and Ahearn, 1963) in Douglas Lake, Michigan, revealed 12 species in surface and deep water samples. These included Candida parapsilopsis, C. pulcherrima, Cryptococcus albidus, Cr. diffluens, Cr. gastricus, Cr. laurentii, Rhodotorula glutinis, R. piliminae, R. rubra, Trichosporon cutaneum, Debaryomyces sp. and 'black yeasts'. In two regions of surface sampling, the population densities averaged 40 and 6 cells per 100 ml respectively, whereas the average deep water count was 40 cells per 100 ml. Yeasts of the genus Rhodotorula predominated.

The predominant genera in Lake Michigan (Hedrick et al, 1964) were Hansenula and Hanseniaspora, Cryptococcus, Torulopsis and Rhodotorula. The average count in this lake was 50/100 ml, the number of yeasts in surface water being less than in sub-surface waters. They reported a lower density of yeasts in Lake Erie but a larger variety of yeasts. Sixteen species of yeast were isolated from mud samples of Lake Michigan (Hedrick and Soyugenc, 1965). They found Cr. laurentii, which is not a typical soil yeast. present in both mud and water samples, but Cr. diffluens and Cr. albidus present only in water samples. The "black

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yeasts' were not recovered from the mud samples; however, they were commonly isolated from lake water collected at the same station.

The same authors (Hedrick and Soyugenc, 1967) reported the isolation of yeasts and moulds from 27 stations in Lake Ontario. They were searching for one or more organisms which might reflect an increase in pollution as measured by increased dissolved solids. However, stepwise multiple correlation analyses did not reveal any consistent association of any one parameter with the distribution of the two most numerous species, Candida guilliermondi and Rhodotorula rubra. However, near the lake bottom, soluble phosphate gave high correlation values for the yeast C. guilliermondi; the parameter, dissolved solids, gave the highest correlation for the yeast R. rubra. At the surface, for both species, there was quite a high degree of correlation between the number of coliforms and the number of yeasts. Other parameters which showed some associations with the yeast distribution of both species, were nitrate-N and dissolved oxygen.

The genera, and number of species of each (in brackets) isolated by these workers were: Candida (4), Cryptococcus (4), Hansenula (1), Rhodotorula (6), Sporobolomyces (1), Torulopsis

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(1), and Trichosporon (2). The moulds included: Alternaria, Aspergillus, Cladosporium, Fusarium, Mucor, Neurospora, and Penicillium. (These moulds are also common terrestrial types - D.W.)

For the L. Ontario stations, the average number of yeasts isolated per 100 ml was: 10 for water at 1 meter, 130 for water at mid-depth and 460 for water near the bottom, and sediment, 46; the respective values for moulds were 6, 16, 16 and 11.

Meyers et al (1970) found the highest concentration of yeasts in the surface waters of Lake Champlain. This lake was described as a "deep, and comparatively unpolluted northern lake", with a pulp and paper mill discharging wastes and heated effluents into one area. The most widespread yeasts, based on the total number of stations, were Cr. albidus, R. rubra, R. glutinis and A. pullulans.

Cell concentrations generally were less than 20 cells/100 ml and varied between 1 to over 400/100 ml. Maximal densities (abou t 200-400 cells per 100 ml) occurred during two successive winter samplings, occasionally under iced conditions. According to the authors, the peak densities during the colder months may have been affected by conditions of the fall and spring turnover. They concluded that

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"... the standing crop of yeasts in the lake is independent of continuous seeding from terrestrial, soil and vegetation sources...".

Ahearn et al (1968) found that yeast populations in saline and fresh waters of urbanized Miami regions reached 10^4 cells/100 ml. The species occuring most frequently in fresh water were found to be Candida tropicalis and Candida krusei. Torulopsis glabrata and Candida albicans, which are classed as obligate saprophytes of man and other warm-blooded animals, were isolated from canals receiving raw sewage.

Earlier, (Cooke et al, 1960) had made a detailed study of polluted water, sewage, and sewage treatment processes revealing 30 yeast species, the yeasts occurring in some habitats in large numbers. A number of these could

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be considered secondary pathogens in humans.

Two recent studies in Canada are concerned with pollution in rivers. Simard and Blackwood (1971) making a study of the St. Lawrence River at stations between Montreal and Quebec obtained counts of 9500 c.f.u./100 ml consistently in some stations. The average count was 725/100 ml including non-polluted stations. They state that the predominance of the red yeasts from July through September is noticeable at all stations with the exception of the high number of Candida found in the samples taken at Quebec City. They believe that the red yeast blooms are favoured by the metabolic conditions existing in the river after the bacteria have degraded the easily metabolizable constituents in the raw sewage.

The South Saskatchewan River was sampled (Spencer et al, 1970) to determine the types and numbers of yeasts contributed to it by the city of Saskatoon. Counts ranging from 0 to 58 c.f.u. per 100 ml at stations above and within the city were observed, increasing below the city as much as 60-fold because of the influx of effluent from an experimental sewage lagoon.

Woollett and Hedrick (1970) have attempted to characterize the yeast population according to the type of

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pollution. Hence, their findings in 13 polluted freshwater habitats reveal that Rhodotorula and Cryptococcus isolates were most numerous at low pollution levels. In locations with heavy industrial waste pollution, Rhodotorula and Candida species were predominant, while in habitats with heavy domestic waste pollution, Candida spp. were the major group, with Rhodotorula spp. of secondary importance. The presence of human wastes was especially associated with large increases in the population of Candida yeasts.

They also found that a higher percentage of yeasts found in non-polluted water utilized nitrate as the sole nitrogen source.

The information given in the reports cited above is of help in interpreting the data collected in the Sudbury lakes. As Meyers et al found for the comparatively unpolluted Lake Champlain, the four predominant species in the Sudbury lakes were Cr. albidus, R. rubra, R. glutinis and A. pullulans. In the Sudbury lakes, Cr. laurentii is also important. However, Candida isolates were few in number as expected in view of the lack of domestic wastes.

The total number of species of yeasts will not be definitely known until all isolates have been identified to

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the species level. However, the number is expected to be at least as high as that reported for Lake Ontario (19 species). The total counts were also indicative of a low pollution level and generally were well under 200 c.f.u./100 ml for all stations. Counts greater than this were encountered only rarely.

Some support can also be given to the observation by others, mentioned above, that yeasts indigenous to an oligotrophic environment are generally those which assimilate nitrate as the nitrogen source. Of the five most widespread species in these lakes, R. rubra and Cr. laurentii do not utilize nitrate.

These results indicate, therefore, that a normal yeast flora is present. It seems reasonable to suppose that the lack of organic nutrients, alone, could account for the decreased populations. Whether or not heavy metals have any additional effect on these organisms has not been investigated. Certainly, yeasts and moulds are more acidophilic than are bacteria and would tolerate the condition of the acid lakes very well.

This reservoir of yeasts could respond at any time to an elevated nutrient level, from organic matter entering the lakes, or, hopefully, by increased bacterial acitivity in the sediments following lime treatment. Nutrient would then be more readily available to the yeasts.

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APPENDIX

- ACKNOWLEDGEMENTS-

The authors' appreciation is expressed for the technical assistance of Tony De Fabrizio, Kathy McGibbon, Tibor Lovasz and Jim Pappas, and for the co-operation of Water Quality Branch personnel in sampling.

APPENDIX

TABLE 1 - A SUMMARY OF PHYSICAL-CHEMICAL CHARACTERISTICS OF RESTORATION LAKES.

PARAMETER	CLEARWATER	LOHI	HANNAH	MIDDLE
Treated (X)		X		X
Initial pH	4 - 4.5	4 - 4.5	4 - 4.5	4 - 4.5
Final pH	4 - 4.5	7 - 7.5	4 - 4.5	7 - 7.5
p.p.m. Ni (initial)	1.1	1.1	1.6	1.1
p.p.m. Ni (final)	1.1	0.26	1.6	0.26
p.p.m. Cu (initial)	0.5	0.5	0.9	0.5
p.p.m. Cu (final)	0.5	0.03	0.9	0.03
Alkalinity (initial)	0	0	0	0
Alkalinity (final)	0	9	0	9
p.p.m. $SO_4^{=}$ (no change)	27	27	56	46
Chlor. *a* (no change)	0.4 - 2.0	0.5 - 2.0	0.6 - 1.6	0.4 - 1.8
Nutrients (before liming) p.p.m.:				
Total P	.008	.013	.01	.008
Free NH_3	.04	.09 - .50 (surface - bottom)	.05	.05
Total N	.12	.23 - .95	.20	.22
NO_3-N	.10	.06	.70	.45
Ca	6	6	12	10
Mg	3	3	5	5
K	1.3	1.2	2	2
D.O.	8.4	8.4 (surf.)	8.2	8.3
sediment pH	5.5	6.0	4.5	4.8

TABLE 2 - A CHEMICAL DATA - MACLEAN LAKE

PARAMETER	DEEP WATER STATION
pH	6.8 - 7.0
chlor. *a* (p.p.m.)	16
Alkalinity	21
Hardness	25
Total P	0.15
Total N	1.0
Conductivity	56

TABLE 3 - A

LOHI LAKE

COUNT PER 100 ml H_2O or PER GRAM SEDIMENT (WET WT.)

SAMPLING DATE	STATION	TOTAL HETEROTROPHIC BACTERIA	TOTAL COLIFORM	TOTAL COLIFORM	FECAL STREP.	YEAST	MOULD	SULFATE REDUCERS	SULFUR OXIDIZERS Thiobacillus thiooxidans	T. thioparus	NITRIFIER NH_3 oxid.
August 2	Surface	3400	-	-	-	28	14	N.D.*	21	210	-
	Depth	1200	-	-	-	36	8		7	93	-
	Sediment	-	-	-	-	-	-		4600	11000	-
September 5	Surface	720	<4	<4	<4	410	<4	3	4	<3	<3
	Depth	910	<4	<4	<4	24	4	240	<3	<3	<3
	Sediment	131000	-	-	-	<10	40	<30	43	90	<30
October 6	Surface	220	<4	<4	<4	4	54	-	-	-	-
October 16	Surface	8700	44	<4	<4	120	14	150	1100	1100	<3
	Depth	6800	96	<4	<4	44	16	150	75	1100	<3
	Sediment	350000	<100	<100	<100	<10	160	4300	2400	11000	<30
October 25	Surface	17000	<4	<4	<4	<2	<2	-	<3	<3	-
	Depth	9100	<4	<4	<4	2	<2	-	<3	<3	-
November 6	Surface	60000	-	-	-	10	12	<3	4	43	<3
	Depth	54000	-	-	-	<2	12	<3	<3	43	-
	Sediment	270000	-	-	-	-	-	>11000	2400	4600	-

ND* _ NOT DETECTED (isolation medium pH 5.5)

TABLE 4 - A

CLEARWATER LAKE

COUNTS PER 100 ml WATER or PER GRAM SEDIMENT (Wet)

SAMPLING DATE	STATION	TOTAL HETEROTROPHIC BACTERIA	TOTAL COLIFORM	FECAL COLIFORM	FECAL STREP.	YEAST	MOULD	SULFATE REDUCERS	SULFURE OXIDIZERS Thiobacillus thiooxidans	SULFURE OXIDIZERS T. thioparus	NITRIFIER NH_3 oxid.
August 2	Surface	170	-	-	-	70	50	ND*	9	210	-
	Depth	420	-	-	-	130	60	"	7	150	-
	Sediment	-	-	-	-	-	-	"	2400	4600	-
September 5	Surface	-	<4	<4	<4	32	10	ND	<3	<3	<3
	Depth	-	<4	<4	<4	680	-	"	7	<3	-
	Sediment	130000	-	-	-	-	-	"	430	<30	<30
October 6	Surface	1400	12	<4	<4	-	-	-	-	-	-
October 16	Surface	5500	4	<4	<4	80	8	<3	<3	<3	<3
	Depth	2600	46	<4	<4	140	12	3	4	<3	<3
	Sediment	270000	200	<100	<100	<10	120	70	90	230	<30
October 23	Surface	500	<4	<4	<4	<2	<2	-	-	-	<3
	Depth	1000	<4	<4	<4	6	<2	-	-	-	<3
November 6	Surface	13 000	-	-	-	14	4	<3	4	<3	-
	Depth	200	-	-	-	<2	2	<3	<3	<3	-
	Sediment	140000	-	-	-	-	-	9	36	1500	-

ND* - NOT DETECTED (isolation medium pH 5.5)

TABLE 5 - A

HANNAH LAKE COUNT PER 100 ml H_2O or PER GRAM SEDIMENT (WET)

SAMPLING DATE	STATION	TOTAL HETEROTROPHIC BACTERIA	TOTAL COLIFORM	FECAL COLIFORM	FECAL STREP.	YEAST	MOULD	SULFATE REDUCERS	SULFUR OXIDIZERS Thiobacillus thiooxidans	SULFUR OXIDIZERS T. thioparus	NITRIFIER NH_3 oxid.
September 6	Surface	420	<4	<4	<4	60	8	<3	<3	<3	<3
	Depth	380	<4	<4	<4	44	16	<3	23	<3	<3
	Sediment	430000	-	-	-	20	40	<30	4600	<300	<30
October 19	Surface	2800	<4	<4	<4	52	14	3	23	4	<3
	Depth	1700	<4	<4	<4	46	18	<3	9	280	-
	Sediment	89000	<100	<100	<100	8	54	<30	930	430	<30
October 23	Surface	540	<4	<4	<4	6	14	-	-	-	-
	Depth	300	<4	<4	<4	16	16	-	-	-	-
November 5	Surface	450	-	-	-	<2	20	4	23	4	-
	Depth	110	-	-	-	<2	14	<3	<3	4	-
	Sediment	92000	-	-	-	3	12	<30	230	2400	-

TABLE 6 - A

MIDDLE LAKE COUNT PER 100 ml H_2O or PER GRAM SEDIMENT (WET)

SAMPLING DATE	STATION	TOTAL HETEROTROPHIC BACTERIA	TOTAL COLIFORM	FECAL COLIFORM	FECAL STREP.	YEAST	MOULD	SULFATE REDUCERS	SULFUR OXIDIZERS		NITRIFIER
									Thiobacillus thiooxidans	T. thioparus	NH_3 oxid.
September 6	Surface	-	-	-	-	-	-	-	-	-	-
	Depth	340	<4	<4	<4	190	24	<3	<3	<3	<3
	Sediment	275000	-	-	-	9	300	<30	4600	-	<30
October 6	Surface	770	<4	<4	<4	44	6	-	-	-	-
October 19	Surface	>150000	8	<4	<4	48	12	4	39	1100	<3
	Depth	56000	<4	<4	<4	28	10	4	<3	9300	-
	Sediment	160000	<100	<100	<100	20	450	930	90	2400	<3
October 23	Surface	>150000	4	<4	<4	8	8	4	<3	460	<3
	Depth	>150000	<4	<4	<4	40	2	<3	<3	>1100	-
November 6	Surface	120000	-	-	-	8	4	4	29	23	-
	Depth	80000	-	-	-	4	12	4	23	14	-
	Sediment	170000	-	-	-	32	80	4600	<300	46000	-

TABLE 7 - A

MAC LEAN LAKE COUNT PER 100 ml H_2O or PER GRAM SEDIMENT (WET)

SAMPLING DATE	STATION	TOTAL HETEROTROPHIC BACTERIA	TOTAL COLIFORM	FECAL COLIFORM	FECAL STREP.	YEAST	MOULD	SULFATE REDUCERS	SULFUR OXIDIZERS		NITRIFIER
									Thiobacillus thiooxidans	T. thioparus	NH_3 oxid.
September 24	Surface	2000	-	-	-	16	36	7	<3	460	<3
	Depth	7300	-	-	-	36	16	4	<3	240	-
	Sediment	-	-	-	-	-	-	-	-	-	-
October 6	Surface	4900	90	<10	<4	28	32	4	-	93	<3
	Sediment	-	-	-	-	-	-	<300	<30	210	230
October 25	Surface	156000	40	<4	<4	4	8	4	<3	460	<3
	Depth	8000	210	<4	<4	10	20	9	<3	23	-
	Sediment	-	-	-	-	44	50	90	230	210	930
November 1	Surface	6900	3100	<4	<4	<4	4	-	-	-	-
	Depth	8400	3700	<4	8	<4	8	-	-	-	-
	Sediment	210000	200	<100	<100	-	-	-	430	210	930

TABLE 8 - A

SUDBURY BEAVER POND pH 6.6 - 7.0

COUNT PER 100 ml H_2O or PER GRAM SEDIMENT (WET)

SAMPLING DATE	STATION	TOTAL HETEROTROPHIC BACTERIA	TOTAL COLIFORM	FECAL COLIFORM	FECAL STREP.	YEAST	MOULD	SULFATE REDUCERS	SULFUR OXIDIZERS		NITRIFIER NH_3 oxid.
									Thiobacillus thiooxidans	T. thioparus	
September 6	Surface	-	-	-	-	-	-	>>1100	>1100	>1100	-
October 4	Surface	-	-	-	-	920	30	1100	-	1100	-
October 24	Surface	15000	100	4	4	440	70	24000	9	1100	210

	DATE DUE		